

**Exhibit A**

**“Marked Up” Amendments to Specification Pursuant to Rule 1.121(b)**

Replace the first full paragraph on page 3 of the Specification with the following:

Also part of this invention is a modified phytase with a specific activity improved over the specific activity of the corresponding unmodified phytase (for example *Aspergillus fumigatus*) wherein the amino acid sequence of the corresponding unmodified phytase has been changed by one or more of deletion, substitution and addition by one or more amino acids to obtain the amino acid sequence of the modified phytase. A preferred phytase has an amino acid sequence homologous to that of the phytase of *Aspergillus niger* (SEQ ID NO:1) [[SEQ ID NO. 1]] and has an amino acid sequence that has been changed in at least one amino acid position selected from the following amino acid positions which correspond to positions of the amino acid sequence of the phytase of *Aspergillus niger*: 27, 66, 71, 103, 140, 141, 188, 205, 234, 238, 274, 277, 282, 340 and 424, in particular wherein the amino acid position is selected from 27, 66, 140, 205, 274, 277, 282, and 340.

Replace the fourth full paragraph on page 3 of the Specification with the following:

Particular modified phytases of this invention are characterized by at least one of the following changes in amino acids at positions: Q27L, Q27N, Q27T, Q27I, Q27V, Q27A, Q27G, S66D, S140Y, D141G, A205E, Q274L, G277D, G277K, Y282H and/or N340S. [[SEQ ID Nos: 10-25]].

Replace the third full paragraph on page 4 of the Specification with the following:

Figure 1: Primary sequence alignment of *A. niger* (*ficuum*), (SEQ ID NO:1) [[SEQ ID NO. 1]] *A. terreus* cbs116.46 (SEQ ID NO:2) [[SEQ ID NO. 2]] and *A. fumigatus* [ATCC 13073] (SEQ ID NO:3) [[SEQ ID NO. 3]] phytase. Stars show identical residues within the active site and rectangles, non-identical residues within the active site.

Replace the sixth full paragraph on page 4 of the Specification with the following:

Figure 4: Complete coding sequence and encoded amino acid sequence of the *Aspergillus nidulans* phytase (SEQ ID NOs:4-6) [[SEQ ID NO.4]].

Replace the last paragraph on page 4 of the Specification with the following:

Figure 5: Complete coding sequence (SEQ ID NO: 7) *[[SEQ ID NO.5]]* and encoded amino acid sequence (SEQ ID NOs:8-9) *[[SEQ ID NO.6]]* of *Talaromyces thermophilus* phytase.

Replace the first full paragraph on page 5 of the Specification with the following:

Figure 6: Complete coding sequence (SEQ ID NO:10) *[[SEQ ID NO. 7]]* and encoded amino acid sequence (SEQ ID NOs:11-12) *[[SEQ ID NO. 3]]* of *Aspergillus fumigatus* [ATCC 13073] phytase.

Replace the second full paragraph on page 5 of the Specification with the following:

Figure 7: Complete coding sequence (SEQ ID NO:13) *[[SEQ ID NO. 9]]* and encoded amino acid sequence (SEQ ID NOs:14-15) *[[SEQ ID NO. 2]]* of *Aspergillus terreus* CBS 116.46 phytase.

Replace the third full paragraph on page 6 of the Specification with the following:

Figure 14a: Primer sets A-N (SEQ ID NOs:24-65) used for site directed mutagenesis.

Replace the fourth full paragraph on page 6 of the Specification with the following:

Figure 14b: Primer sets O-T (SEQ ID NOs:66-77) used for site directed mutagenesis.

Replace the third full paragraph on page 7 of the Specification with the following:

Figure 23: Natural variation of phytases in different isolates of *A. fumigatus* [ATCC 13073].  
The predicted protein sequences (SEQ ID NOs:78-82) are shown and compared to that of the phytase from *A. fumigatus* strain ATCC 13073. Only the amino acids which differ from those in #13073 are shown.

Replace the paragraph that begins on page 10 and ends on page 11 of the Specification with the following:

Although other three-dimensional phytase structures may be obtained and used, it is preferred to use the three-dimensional of the *Aspergillus niger* phytase in the process of this invention (see Kostrewa et al., *Nature Structural Biology* 4:185 (1997)) or of *Aspergillus fumigatus*. A useful strain of *Aspergillus niger* may be obtained from the American Type Culture Collection [address] under accession number ATCC 9142. Like any three-dimensional phytase structure useful in this invention, the three-dimensional structure of the *A. niger* phytase is obtained by techniques known to a skilled practitioner. Based on an amino acid sequence such as the *A. niger* amino acid sequence provided herein, (SEQ ID NO:1) [[SEQ ID NO. 1]] computer programs can provide theoretical structures. Crystal structures can also be obtained, as

in Example 1 below. From these three-dimensional structures, active sites can be defined, such as the part of the phytase which interacts with substrate. This active site can then be localized to the segment or segments of the amino acid sequence which together form the active site, which segment or segments can then be modified, the whole sequence expressed as a modified phytase which is then tested to see if the activity has been improved. By this means a desired property can be designed into an unmodified phytase, using the three dimensional structure of the *A. niger* phytase as a template based on the alignment.[.]

Replace the last paragraph on page 12 of the Specification with the following:

It is also an object of the present invention to provide a phytase as specified above which is characterized by at least one of the following mutations: Q27L, Q27N, Q27T, Q27I, Q27V, Q27A, Q27G, S66D, S140Y, D141G, A205E, Q274L, G277D, G277K, Y282H and/or N340S. *[[SEQ ID Nos. 10-25]]*

Replace the first full paragraph on page 13 of the Specification with the following:

It is furthermore an object of the present invention to provide phytase muteins which are resistant against degradation by proteases of fungal, preferably *Aspergillus* and most preferably *Aspergillus niger* (ficcum) origin. Such muteins are characterized therein that at least [at] one of the following positions (which refers to the homologous position in the amino acid sequence of *A. niger*), namely position 130 or 129 and 130, preferably of the *Aspergillus fumigatus* or 167, 168 preferably of the *A. nidulans* phytase amino acid sequence, the amino acid

In re Divisional Application of : Dirk KOSTREWA, *et al.*  
Parent U.S. Serial No.: 09/044,718  
For: **MODIFIED PHYTASES**

which is present in the wild type sequence has been replaced against another amino acid which is known to change the protease sensitivity, e.g. in the case of *A. fumigatus* at position 130 from "S" to "N" [[SEQ ID NO 26]] and at position 129 from "R" to "L" [[SEQ ID NO. 27]] and in case of *A. nidulans* at position 167 from "K" to "G" [[SEQ ID NO 28]] and at position 168 from R to Q. [[SEQ ID NO. 29]] Such positions can be also combined with those providing for improved activity properties.

Replace the first full paragraph on page 24 of the Specification with the following:

Primer #39:

BspHI  
5' TAT ATC ATG ATT ACT CTG ACT TTC CTG CTT TCG 3' (SEQ ID NO:16)  
M I T L T F L L S (SEQ ID NO:17)

Replace the second full paragraph on page 24 of the Specification with the following:

Primer #40:

EcoRV  
3' CCT CTC ACG AAA TCA ACT CTA TAG ATA TAT 5' (SEQ ID NO:18)  
G E C F S \* (SEQ ID NO:19)

Replace the first full paragraph on page 25 of the Specification with the following:

In re Divisional Application of : Dirk KOSTREWA, *et al.*  
Parent U.S. Serial No.: 09/044,718  
For: **MODIFIED PHYTASES**

Fum28:

5' ATATATCGGCCGAGTGTCTGCGGCACCTAGT 3' (SEQ ID NO:20)  
EagI

Replace the second full paragraph on page 25 of the Specification with the following:

Fum11:

5' TGAGGTCATCCGCACCCAGAG 3' (SEQ ID NO:21)

Replace the third full paragraph on page 25 of the Specification with the following:

Fum26:

5' CTAGAATTCATGGTGACTCTGACTTTCCTGCTTTCGGCGGCGTATCTGCTTTCC 3'  
(SEQ ID NO:22)

Replace the third full paragraph on page 25 of the Specification with the following:

Fum27:

5' GGCCGAAAGCAGATACGCCGCCGAAAGCAGGAAAGTCAGAGTCACCATGAATT  
3' (SEQ ID NO:23)

In re Divisional Application of : Dirk KOSTREWA, *et al.*  
Parent U.S. Serial No.: 09/044,718  
For: MODIFIED PHYTASES

## **Exhibit B**

### **“Marked Up” Amendment to Claim Pursuant to Rule 1.121(c)**

15. (Amended) A modified phytase with a specific activity improved over the specific activity of the corresponding unmodified phytase wherein the amino acid sequence of the modified [unmodified] phytase has one or more of the following mutations selected from the group consisting of S66D, S140Y, D141G, A205E, Q274L, G277D, G277K, Y282H and N340S.

2020-03-20 10:06:24